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Flow-Mediated Vasodilation of the Femoral and Brachial Artery Induced by Exercise in Healthy Nonsmoking and Smoking Men

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OBJECTIVES We sought to analyze diameter changes of conduit arteries in response to whole-body exercise and hypothesized that this response might be endothelium-dependent and, therefore, impaired in smokers.

BACKGROUND Hyperemia and coincident vasodilation are pivotal mechanisms for meeting the increased metabolic demands of active muscle tissue during physical exercise, but studies in humans are sparse.

METHODS We studied diameter and blood flow of the femoral and brachial arteries in response to a submaximal bicycle exercise test in 10 nonsmoking and 8 smoking healthy male subjects. During an exercise period of 40 min the investigated conduit arteries were periodically scanned in longitudinal sections by high-resolution ultrasound. In the same subjects flow-mediated dilation (FMD) of the brachial artery was recorded by inducing an ischemia through a forearm-occluding cuff.

RESULTS In response to exercise the diameter of the femoral artery significantly increased in both nonsmokers and smokers, with a diminished response in smokers (9.2 ± 1.9% vs. 4.8 ± 1.6%, $p < 0.001$). Flow-mediated dilation of the brachial artery induced by forearm occlusion was also reduced in smoking subjects, revealing a strong correlation between these different methods of FMD (exercise vs. forearm ischemia) ($r = 0.88$, $p < 0.001$). In contrast, blood flow increase of the femoral artery was similar in nonsmoking and smoking subjects (392 ± 77% vs. 382 ± 109%, p = NS).

CONCLUSIONS Conduit arteries react with a flow-mediated dilation in response to whole-body exercise. The impairment of this vasodilation observed in smokers was strongly related to a decrease of endothelium-dependent dilation induced by forearm ischemia, indicating that endothelial dysfunction represents the underlying mechanism. (J Am Coll Cardiol 2001;38: 1313–9) © 2001 by the American College of Cardiology

Physical exercise markedly raises blood flow to skeletal muscles to meet the metabolic demands of active muscle tissue (1). A crucial mechanism responsible for the increase in blood flow is vasodilation (2), occurring not only in local resistance vessels (3) but also in proximal conduit arteries (4,5). Our knowledge about this exercise-induced vasodilation is sparse and based on results of animal studies (4) and hemodynamic investigations in humans (2). So far, direct measurements of the vessel diameter over time and in response to exercise are limited to a single study investigating vascular response to handgrip exercise (6). Corresponding data about diameter changes of human conduit arteries in response to whole-body exercise are not yet available.

In contrast, the evaluation of flow-mediated dilation (FMD) induced by reactive hyperemia following the release of a forearm-occluding cuff is an established method for assessing endothelial function (7–9). It has been convincingly demonstrated that FMD is endothelium-dependent and mediated by nitric oxide (10,11).

In this study we investigated the diameter changes of the femoral and brachial arteries in response to a submaximal steady-state bicycle exercise in smoking and nonsmoking subjects. In addition, we assessed endothelium-dependent FMD induced by a temporary forearm ischemia in order to reveal potential analogies between these different kinds of flow-mediated vasodilation.

METHODS

Subjects. Eighteen healthy men, age 31 to 50 years, participated in the study. Ten subjects were nonsmokers and eight subjects were smokers with a daily consumption of 20 or more cigarettes per day for at least five years. All subjects were free of diabetes, thyroid disease, dyslipemia, hypertension or family history of premature vascular disease. None of them were taking any cardiovascular medication or antioxidant agents. Physical activity was assessed in a detailed interview with special regard to the amount of time spent per week in performing moderate and vigorous exercise. The subjects were recruited from university staff members. Written informed consent was obtained from each subject after a detailed description of the procedure.

Study protocol. The subjects were examined in our laboratory on two separate days. All vascular studies were performed by the same investigator, experienced in ultra-

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Abbreviations and Acronyms
ANOVA = analysis of variance
ECG = electrocardiogram
FMD = flow-mediated dilation

sound with special training in endothelial function studies, in a temperature-controlled room (20°C to 22°C) at the same time of the day (between 1 and 2 PM) to avoid potential confounding influences such as diurnal variation of endothelial function (12,13). All subjects had eaten a typical continental breakfast and refrained from further food intake and smoking thereafter. On the first day, the diameter and blood flow of the femoral and brachial arteries were measured in response to submaximal steady-state exercise by high-resolution ultrasound with the use of a 13.0-MHz linear-array transducer and a standard Acuson Sequoia 512 system (Acuson, Mountain View, California). On the second day, an ultrasound technique, as described by Celermajer et al. (7), was applied to study endothelium-dependent and -independent dilation of the brachial artery. Standard laboratory assays were used to determine fasting lipid levels.

Assessment of exercise-induced changes in diameter and blood flow of the femoral and brachial arteries. The exercise test was performed on a standard bicycle ergometer (Ergometrics 900, Marquette-Hellige, Freiburg, Germany). Subjects adopted a conventional cycling posture. The exercise test was started with a constant workload of 100 W for 20 min. Thereafter the workload was increased to 150 W for another 20 min. The pedaling cadence was kept constant between 70 and 80 rev/min.

B-mode scans of the right brachial artery were obtained in a longitudinal section 5 to 10 cm above the elbow while the subject placed his arm in an extended position on the handlebars. Because arm movements could be limited to a minimum, it was possible to obtain appropriate images although the subjects continued cycling. Scans of the right femoral artery were obtained 3 to 5 cm distal to the bifurcation of the common femoral artery. The moving legs made simultaneous measurements in this region impossible. Therefore a short break of exercise was necessary to obtain appropriate images. Scans of the femoral artery were taken within 30 s with the right leg almost completely extended from the groin (about 10° of flexion).

Photographic images of both arteries were obtained at rest after subjects' sitting for 10 min on the ergometer. During submaximal bicycle exercise images of the brachial and femoral arteries were repeatedly recorded in intervals of 3 and 5 min, respectively. Immediately after the test the subjects remained in the sitting position for another 30 min with the right leg extended to obtain postexercise images in 3-min intervals. The heart rate was continuously recorded with an electrocardiogram (ECG) lead integrated in the ultrasound system. Blood pressure was measured manually in 5-min intervals throughout the study.

To ensure consistency of the images with serial scans the transducer position was marked on the skin and measurements were obtained at a fixed distance from an anatomic marker such as a bifurcation. When the lumen/arterial wall interface was adequately visualized, three ECG-gated end-diastolic B-mode images were acquired and stored. Thereafter the velocity of arterial flow was recorded with a pulsed Doppler signal at a 70° angle to the vessel.

The vessel diameter was measured from the anterior to the posterior interface between the media and adventitia ("m" line) (14). The images of an individual subject were analyzed by only one investigator who was blinded to the subjects' smoking status and the stage of the study to avoid interobserver variability. To determine intraobserver variability the baseline values were compared with those of the second experimental day. The coefficients of variation of baseline brachial and femoral artery diameter were 2.9% and 2.7%, respectively. Exercise-induced changes in artery diameter and blood flow were expressed as a percentage change of baseline values. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle) by the heart rate and the cross-sectional area of the vessel (πr^2).

Measurement of endothelium-dependent and -independent dilation of the brachial artery. Endothelium-dependent and -independent dilation of the brachial artery was determined as described in detail by Celermajer et al. (7). Briefly, end-diastolic diameter and flow velocity measurements as described above were performed in the brachial and femoral arteries at rest in the sitting and in the supine position 10 min later. Thereafter a forearm cuff was inflated at 250 mm Hg for 4 min, followed by pressure release. Measurements of the brachial artery diameter were performed at 45 to 90 s after deflation, following the recording of flow velocity for the first 15 s of reactive hyperemia. A period of 10 min was allowed for recovery of the vessel. Then 400 μg of glyceryl trinitrate was sublingually administered to induce endothelium-independent dilation. A final scan was performed 3 to 4 min later. Endothelium-dependent and -independent dilation was determined as the percentage change in diameter to the mean value of the three baseline measurements. Reactive hyperemia was calculated as the maximal flow in the first 15 s after cuff deflation divided by the baseline flow.

Statistical analysis. All analyses were performed using the statistical software package Graph Pat Prism (version 3.0, San Diego, California). Data are presented as mean values ± standard deviation. All variables were distributed normally as shown by Kolmorogov-Smirnov statistics. The vessel diameters and blood flow values obtained by serial measurements during exercise were evaluated for trends over time by repeated measures analysis of variance (ANOVA). Significant findings ($p < 0.05$) were further analyzed by the Student-Newman-Keuls post-hoc test. Linear regression analyses were performed for the changes of vessel diameter

Table 1. Clinical Characteristics and Fasting Lipid Levels of the Study Groups

	Nonsmokers (n = 10)	Smokers (n = 8)	p Value
Age (yrs)	39.6 ± 5.8	38.9 ± 4.1	NS
Body mass index (kg/m^2)	24.4 ± 1.5	24.1 ± 0.8	NS
Heart rate (beats/min)	71 ± 7	72 ± 6	NS
Systolic blood pressure (mm Hg)	120 ± 6	126 ± 6	NS
Moderate physical activity (h/wk)	1.7 ± 0.8	1.6 ± 1.1	NS
Total cholesterol (mg/dl)	193 ± 33	182 ± 29	NS
LDL cholesterol (mg/dl)	109 ± 36	107 ± 31	NS
HDL cholesterol (mg/dl)	65 ± 17	54 ± 12	NS
Triglycerides (mg/dl)	92 ± 23	98 ± 31	NS

Data presented are mean values ± SD.
HDL = high density lipoprotein; LDL = low density lipoprotein.

and blood flow during exercise and were compared between study groups. Baseline characteristics and vascular parameters were analyzed by the use of an unpaired or paired Student *t* test where appropriate. For correlation analysis, univariate Pearson's correlation coefficients were calculated. All p values are two-tailed and a value of $p < 0.05$ was considered to be significant.

RESULTS

Clinical and biochemical characteristics. There were no significant differences between nonsmoking and smoking men with respect to clinical characteristics, physical activity and fasting lipid levels (Table 1). In response to submaximal bicycle exercise, systolic blood pressure and heart rate increased from 123 ± 7 mm Hg at rest to 154 ± 5 mm Hg ($p < 0.001$) and from 71 ± 6 beats/min at rest to 135 ± 6 beats/min ($p < 0.001$), respectively. The changes of heart rate and blood pressure were similar between nonsmoking and smoking subjects (heart rate at 150 W workload: 133 ± 7 vs. 136 ± 5, p = NS).

Femoral and brachial artery diameter and blood flow responses to exercise. Baseline femoral artery diameter and blood flow were not different between nonsmoking and smoking subjects. In response to exercise the femoral artery diameter increased significantly in both nonsmokers and smokers (Fig. 1 and Table 2). Exercise-induced dilation of the femoral artery was significantly greater in nonsmokers (9.2 ± 1.9% vs. 4.8 ± 1.6%, $p < 0.001$). This finding was confirmed by linear regression analyzes comparing the changes of femoral artery diameter during exercise between both study groups, with a significantly greater response in

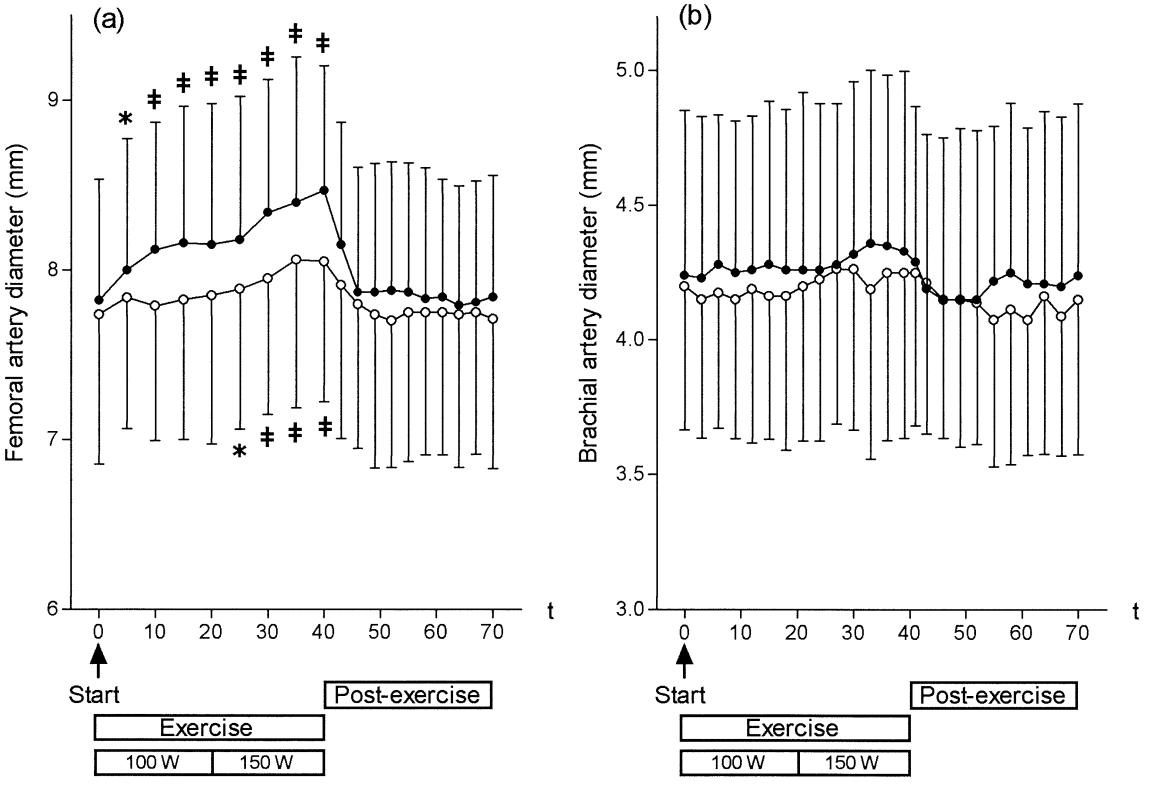

Figure 1. Exercise-induced changes of the femoral **(a)** and brachial **(b)** artery diameter in nonsmoking **(black circles)** and smoking **(white circles)** subjects. **(a)** Evaluation of the femoral artery diameter during exercise by repeated measures analysis of variance (ANOVA) showed significant changes in both nonsmoking and smoking subjects ($p < 0.001$). Comparison of the line plots of both study groups were performed by using linear regression analyses and revealed significantly different slopes of femoral artery diameter increases. **(b)** Evaluation of brachial artery diameter changes during exercise by repeated measures ANOVA gave p values of 0.03 and 0.09 for nonsmoking and smoking subjects, respectively. Comparison of the line plots of both study groups were performed by linear regression analyses and revealed no significant difference between the slopes of brachial artery diameter increases. *$p < 0.05$ and ‡$p < 0.001$ for comparison to baseline.

Table 2. Vascular Baseline and Reactivity Parameters of the Study Groups

	Nonsmokers (n = 10)	Smokers (n = 8)	p Value
Exercise-induced hyperemia			
Baseline diameter FA (mm)	7.8 ± 0.7	7.7 ± 0.9	NS
Exercise-induced dilation FA (%)	9.2 ± 1.9	4.8 ± 1.6	< 0.001
Baseline flow FA (ml/min)	269 ± 74	268 ± 64	NS
Hyperemia FA (%)	392 ± 77	382 ± 109	NS
Baseline diameter BA (mm)	4.2 ± 0.6	4.2 ± 0.5	NS
Exercise-induced dilation BA (%)	4.7 ± 2.9	2.8 ± 2.3	NS
Baseline flow BA (ml/min)	114 ± 28	124 ± 48	NS
Hyperemia BA (%)	236 ± 53	276 ± 153	NS
Forearm ischemia-induced hyperemia			
Baseline diameter BA (mm)	4.3 ± 0.6	4.2 ± 0.5	NS
Flow-mediated dilation BA (%)	7.7 ± 1.4	4.1 ± 1.9	< 0.001
GTN-mediated dilation BA (%)	15.1 ± 3.8	15.2 ± 2.2	NS
Hyperemia BA (%)	498 ± 72	505 ± 77	NS

Data presented are mean values ± SD.
BA = brachial artery; FA = femoral artery; GTN = glyceryl trinitrate.

arterial dilation in nonsmokers. Postexercise the femoral artery diameter returned to baseline values within 6 min in both groups.

In response to exercise there was a rapid increase in femoral artery blood flow within 5 min. The amount of hyperemia at the higher workload of 150 W was nearly the same between nonsmokers and smokers (Fig. 2 and Table 2).

Baseline brachial artery diameter and blood flow was comparable between nonsmoking and smoking subjects. The hyperemic response was lower in the brachial artery compared with the femoral artery (254± 107% vs. 387± 90%, $p < 0.001$) (Fig. 2), but was similar in nonsmokers and smokers (Fig. 2 and Table 2). Exercise-induced dilation of the brachial artery reached borderline significance measured by ANOVA in nonsmoking subjects ($p = 0.03$) and was not statistically significant in smoking subjects ($p = 0.09$).

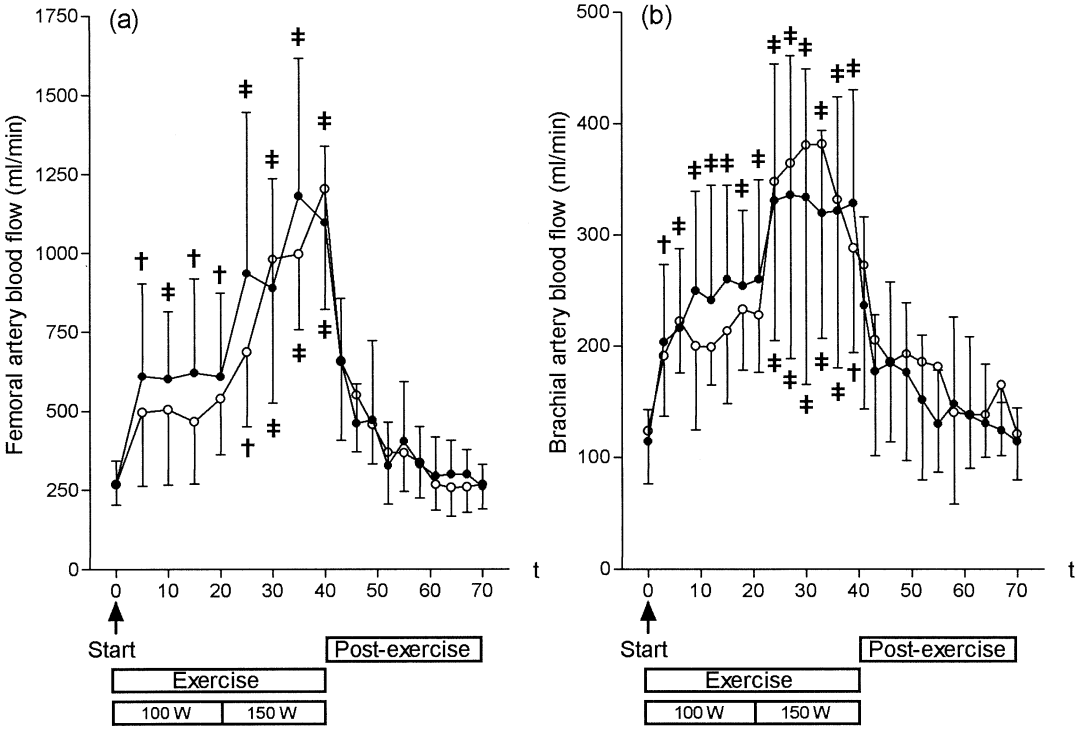

Figure 2. Exercise-induced changes of the femoral **(a)** and brachial **(b)** artery blood flow in nonsmoking **(black circles)** and smoking **(white circles)** subjects. Evaluation of femoral and brachial artery blood flow during exercise by repeated measures analysis of variance showed significant changes in both nonsmoking and smoking subjects ($p < 0.001$). Comparison of the line plots of both study groups were performed by linear regression analyses and revealed no significant difference between the slopes of femoral and brachial artery blood flow increases. *$p < 0.05$, †$p < 0.01$, and ‡$p < 0.001$ for comparison to baseline.

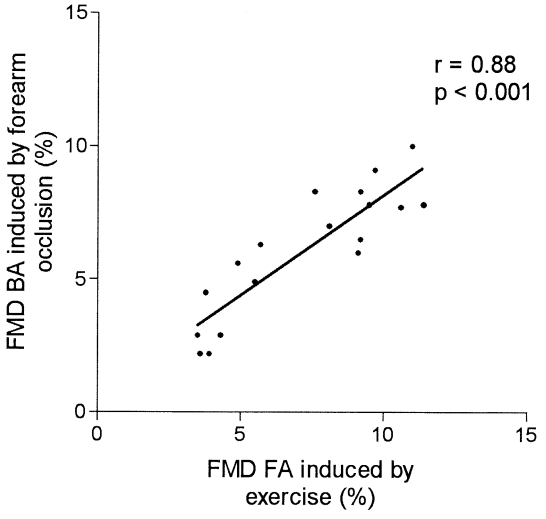

Figure 3. Correlation between flow-mediated dilation of the femoral artery (FMD FA) induced by exercise and flow-mediated dilation of the brachial artery (FMD BA) induced by forearm cuff occlusion.

Endothelium-dependent and -independent dilation of the brachial artery in response to forearm ischemia. Endothelium-dependent FMD of the brachial artery induced by forearm ischemia was significantly reduced in smoking subjects (Table 2). In contrast, endothelium-independent dilation did not differ between the study groups. The baseline flow and the hyperemic response following forearm occlusion were similar in the two groups.

In univariate linear regression analysis the exercise-induced dilation of the femoral artery was strongly correlated with the forearm ischemia-induced endothelium-dependent dilation of the brachial artery ($r = 0.88$, $p < 0.001$) (Fig. 3).

DISCUSSION

This study is the first to describe diameter changes of conduit arteries in response to cycling, which is a dynamic, whole-body exercise recommended in many physical activity programs (15). In response to a submaximal bicycle exercise we observed a dilation of the femoral artery with an increase in blood flow.

Mechanism underlying exercise-induced vasodilation. The initial rapid component of hyperemia is considered to be mediated by direct cell-to-cell conduction in a functional syncytium of the microcirculation that is embedded in the interstitial fluid and subjected to changes in the level of metabolic by-products (3,16). As blood flow in the microcirculation is reported to increase by a factor of two to three, a concomitant dilation of larger conduit arteries located upstream from the site of metabolic exchange is assumed to represent an important vascular response for a further enlargement of blood flow (4,17,18). Because the dilation of microvessels raises blood flow in upstream feed arteries (3), flow-mediated dilation represents a plausible mechanism for vasodilation induced by exercise. Release of nitric oxide represents the central factor responsible for FMD (10,11,19–22). Inhibition of peripheral adrenergic vasoconstriction and release of prostacyclin are other mechanisms that contribute to conduit artery dilation (23,24).

Correlation between flow increase and vasodilation. The observed increases in blood flow and vessel diameter were gradual and dependent on the amount of workload with different steady states at different levels of workload in the investigated arteries (Figs. 1 and 2). Compared with the femoral artery, the increase of the exercise-induced hyperemic response was smaller in the brachial artery, which can be explained by the considerably higher workload of the legs in cycling. Despite a clear rise of blood flow in the brachial artery, the changes in brachial artery diameter were not significant in smokers and reached borderline significance in nonsmokers. Our observations match well with recent data showing the volume of blood flow to be augmented with the intensity of exercise (6,25), as with growing oxygen consumption of working muscle tissue. So far, in a single study Shoemaker et al. (6) reported that high-intensity handgrip exercise results in a diameter increase of the brachial artery whereas low-intensity work does not. The authors speculated that the workload of low-intensity handgrip exercise is too small to provoke a dilation in the upstream conduit arteries. A positive correlation between the increase in blood flow and resulting vasodilation is furthermore described in investigations, shedding light on the mechanisms underlying FMD (26). The exercise-induced increase in blood flow causes endothelial shear stress (27) that is sensed and transduced by the endothelium (26), resulting in vasodilation being proportional to the increase of shear stress (28,29).

Methods of assessing FMD. A noninvasive method of assessing endothelial function is the measurement of FMD of the brachial artery in response to forearm ischemia and was first described in 1992 (7), providing important insights into the risk factors of atherosclerosis (30). This method of inducing increased blood flow by an ischemia-producing forearm occlusion shows an excellent reproducibility (31) and is valid for assessing endothelium-dependent FMD (32). However, it does not represent a physiologic stimulus of FMD because a potential release of ischemic metabolites or a change in the arterial distending pressure due to cuff occlusion could produce arterial dilation (33,34). For this reason, and in order to investigate FMD under physiologic conditions, we used this additional exercise testing to provoke a hyperemic response. In this physiologic situation we revealed that in smoking subjects the exercise-induced dilation of the femoral artery is impaired. Moreover we detected a decline of ischemia-induced dilation of the brachial artery in smokers. The observed significant correlation between these two different kinds of assessing FMD ($r = 0.88$, $p < 0.001$) (Fig. 3) suggests that vascular responses induced by different stimuli could be influenced in a similar way through various factors, as it was proven to be for smoking in our study.

It has been convincingly demonstrated that exercise-induced FMD is endothelium-dependent and mediated by the release of nitric oxide (10,11,19–22,35). Thus, we interpret our finding of an impaired FMD in smokers as evidence for endothelial dysfunction. Both active and passive cigarette smoking were shown to be associated with an impaired endothelium-dependent dilation in a dose-dependent manner (36,37). The mechanisms responsible for this arterial damage remain poorly understood, but free radical components of cigarette smoke are implicated as an important cause (38). Endothelial dysfunction, an important early step in the atherosclerotic process (39), could be the key in the explanation of deleterious cardiovascular effects by cigarette smoking (40) and of its extraordinary role in the pathogenesis of atherosclerotic peripheral arterial disease (41).

Possible physiologic role of conduit artery dilation and clinical implications. One assumed physiologic role of conduit artery dilation is its contribution to the increased blood flow in response to exercise (4,17). Our finding of similar increases in exercise-induced blood flow in both nonsmoking and smoking subjects, despite a significantly reduced FMD in smokers and results of a previous study (42), imply that this contribution may not be of major relevance. We speculate that FMD of conduit arteries represents a regulatory mechanism to counteract the occurrence of increased shear stress and nonlaminar turbulent flow. Impairment of FMD, found in our study to be induced by smoking, could in this way stimulate atherogenesis by increasing physical forces on large conducting vessels (26,43).

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